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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 065691/0214

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): Unassigned 09/786722

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR99/02133	September 8, 1999	September 8, 1998

TITLE OF INVENTION
ANTI-OZF PROTEIN MONOCLONAL ANTIBODIES AND THEIR APPLICATIONS IN THE DIAGNOSTIC AND THERAPEUTIC FIELD

APPLICANT(S) FOR DO/EO/US
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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - ☒ has been transmitted by the International Bureau.
   - ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - ☐ have been transmitted by the International Bureau.
   - ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
11. ☐ Applicant claims small entity status under 37 CFR 1.27 .

Items 12. to 17. below concern other document(s) or information included:

12. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
13. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
14. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☒ Other items or information: Associate Power of Attorney

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) Unassigned 09/786722	INTERNATIONAL APPLICATION NO PCT/FR99/02133	ATTORNEY'S DOCKET NUMBER 065691/0214

18. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO.....$860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482).....$690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2) $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than 20 Months from the earliest claimed priority date (37 CFR 1.492(e))		

Claims	Number Filed		Included in Basic Fee		Extra Claims		Rate		
Total Claims	18	-	20	=	0	×	$18.00	$0.00	
Independent Claims	1	-	3	=	0	×	$80.00	$0.00	
Multiple dependent claim(s) (if applicable)							$270.00	$270.00	
TOTAL OF ABOVE CALCULATIONS =								$1130.00	
Reduction by ½ for filing by small entity, if applicable.								$0.00	
SUBTOTAL =								$1130.00	
Processing fee of $130.00 for furnishing English translation later the 20 months from the earliest claimed priority date (37 CFR 1.492(f). +									
TOTAL NATIONAL FEE =								$1130.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +									
TOTAL FEES ENCLOSED =								$1130.00	

				Amount to be:	
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a. ☒ A check in the amount of $1130.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 19-0741 in the amount of $0.00 to the above fees. A duplicate copy of this sheet is enclosed.

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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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ANTI-OZF PROTEIN MONOCLONAL ANTIBODIES AND THEIR APPLICATIONS IN THE DIAGNOSTIC AND THERAPEUTIC FIELD

The present invention relates to a monoclonal antibody specific for the OZF protein as well as a pharmaceutical composition comprising said antibody, intended for the prevention, treatment or diagnosis of a pathological condition linked to the abnormal expression of the OZF protein such as cancer. The invention comprises, in addition, methods for the detection of the OZF protein as well as methods for the selection of compounds capable of interacting with the OZF protein.

Most proteins having zinc finger motifs are characterized by their capacity to bind to RNAs or DNAs. This zinc finger motif has been found in genes controlling development, genes for transcription factor or genes linked to the formation of tumors, thus demonstrating the importance of this zinc finger structure in regulating the expression of genes.

Among the genes encoding these zinc finger motifs, a gene encoding a protein consisting only of zinc finger motifs, called OZF gene (OZF for "Only Zinc Fingers") has been isolated (Le Chalony et al., 1994) and the corresponding cDNA has been cloned and sequenced. The amino acid sequence of the putative protein encoded by the OZF gene comprises 292 amino acid residues, including 10 zinc finger motifs of 28 amino acid residues, for an estimated molecular weight of 33 kDa. Comparative analysis of the OZF protein has shown that the domain containing the zinc finger structure comprises high homologies with other proteins containing zinc finger motifs.

Moreover, Ferbus et al. (Ferbus et al., 1996) have been able to demonstrate certain characteristics of the OZF protein from a recombinant OZF protein produced in E. coli. In particular, these authors have shown that the recombinant OZF protein was capable of binding zinc ions, DNA and heparin. In addition, these

authors were also able to show, using a polyclonal antibody obtained from a recombinant OZF protein fused with a maltose binding protein (MBP) that the OZF protein was expressed in human mammary cells of the epithelial type, preferably in the nucleus, while it was only weakly expressed in the myoepithelial and stroma cells of this mammary tissue. These authors were also able to demonstrate that the OZF protein was expressed in a tumor cell line established from a pancreatic tumor.

The inventors have demonstrated, surprisingly, using anti-OZF protein polyclonal antibodies, that the OZF protein was overexpressed in primary tumors which exhibited amplification of the OZF gene but also in primary tumors which did not exhibit amplification of the gene.

Moreover, the inventors have also demonstrated that the overexpression of the OZF protein in these primary tumors was restricted to the tumor cells.

These results thus show that the OZF protein appears as a marker for tumor cells.

The production of antibodies capable of specifically recognizing the OZF protein would thus make it possible to detect tumor cells characterized by an abnormal expression of OZF protein.

Anti-OZF protein polyclonal antibodies have already been described (Ferbus et al., 1996). These antibodies were prepared by immunizing rabbits against a recombinant protein obtained by fusion of the OZF protein and the MBP protein, and then purified on an immunoabsorbent consisting of the fused recombinant protein OZF-MBP coupled to a sepharose 4B column. These polyclonal antibodies can thus recognise several epitopes of the OZF protein and in particular some epitopes common to the human or mammalian proteins having zinc finger motifs, given the high sequence homology noted between the OZF protein and other proteins having zinc finger motifs.

The inventors have thus been able to demonstrate nonspecific reactions obtained with certain proteins having zinc finger motifs for these polyclonal antibodies.

While the specificity of these polyclonal antibodies can prove to be sufficient for particular applications such as the detection of OZF protein in isolated homogeneous tissues or cells, their specificity and possibly their affinity could prove to be insufficient as is in fact recognized by the authors of the publication describing these polyclonal antibodies. The authors indeed specify, in their conclusion, that it would be necessary to have available anti-OZF antibodies which make it possible to identify with precision the types of cell expressing the OZF protein. The production of such antibodies, directed against a specific epitope of the human OZF protein, would undoubtedly make it possible not only to identify the types of cell normally or abnormally expressing the OZF protein but also to search for the target, in particular nuclear, nucleic sequences for the OZF protein, and to identify the genes capable of being regulated by the OZF protein.

Thus, a need currently exists for having a monoclonal antibody specifically directed against the human OZF protein. Such an antibody would not only be useful for studying and thus knowing better the role played by the OZF protein in the regulation of genes, but would also be useful in the light of the results presented by the inventors in the present invention, for other applications such as therapeutic or diagnostic applications, in particular *in vivo* or *in vitro* using a heterogeneous biological sample as starting material, or for the targeting of compounds capable of modulating the biological activity of the OZF protein.

The present invention is based on the discovery of the possibility of obtaining, by a particular

preparation approach, a monoclonal antibody characterized by a specificity which has never yet been previously obtained. It has thus been discovered that by immunizing a mouse with a recombinant OZF protein, it is possible to obtain an antibody recognizing a specific epitope of the OZF protein and which does not recognize other mammalian proteins having zinc finger motifs.

Thus, the subject of the present invention is a monoclonal antibody or one of its fragments which is capable of specifically binding to an epitope of the OZF protein, preferably the human OZF protein.

The term epitope in the present description is understood to mean any determinant of the protein responsible for the specific interaction with the antibody. The epitopic determinant usually consists of groups of molecules having chemically active surfaces such as amino acids or side chains of sugars and having a specific three-dimensional structure and/or a characteristic specific charge.

The monoclonal antibody fragments according to the invention comprise any fragment of said monoclonal antibody which is capable of binding to the OZF protein epitope to which binds the monoclonal antibody from which said fragment is derived. Examples of such fragments include in particular single chain monoclonal antibodies or monovalent fragments Fab or Fab' and divalent fragments such as F(ab')2, which possess the same binding specificity as the monoclonal antibody from which they are derived. A fragment according to the invention may also be a single chain Fv fragment produced by methods known to persons skilled in the art and as described for example by Skerra et al., 1988 and King et al., 1991.

According to the present invention, monoclonal antibody fragments of the invention may be obtained from monoclonal antibodies as described above by methods such as digestion with enzymes, such as pepsin

or papain and/or by cleavage of the disulfide bridges by chemical reduction. Alternatively, the monoclonal antibody fragments included in the present invention may be synthesized by automated peptide synthesizers such as those supplied by the company Applied Biosystems, and the like, or may be manually prepared using techniques known to persons skilled in the art and as described for example by Geysen et al., 1978.

In general, for the preparation of monoclonal antibodies or fragments thereof, reference may be made to the techniques which are in particular described in the manual "Antibodies" (Harlow et al., 1988) or to the technique for preparation using hybridomas which was described by Kohler and Milstein in 1975.

The monoclonal antibodies according to the invention may be obtained for example from the cell of an animal immunized against the OZF protein, or one of its fragments, comprising the epitope specifically recognized by said monoclonal antibodies according to the invention. Said OZF protein, or one of its said fragments, may be in particular produced according to the customary procedures by genetic recombination from a nucleic acid sequence contained in the cDNA sequence encoding the OZF protein or by peptide synthesis from an amino acid sequence contained in the peptide sequence of the OZF protein.

The monoclonal antibodies according to the invention may be for example purified on an affinity column on which the OZF protein or one of its fragments comprising the epitope specifically recognized by said monoclonal antibodies according to the invention has been immobilized beforehand.

Preferably, the monoclonal antibody or one of its fragments according to the present invention binds to the linear or conformational epitope of the N-terminal domain of the human OZF protein whose sequence exhibits a low degree of homology compared with the sequences of other proteins having zinc finger motifs.

More preferably, the monoclonal antibody or one of its fragments according to the invention binds to an epitope situated on the first fifteen amino acids of the N-terminal domain of the OZF protein as described by Le Chalony et al., 1994, in figures 1B and 1C, page 400.

Thus, the invention relates to a monoclonal antibody or one of its fragments according to the invention, characterized in that the epitope of the OZF protein is situated on the N-terminal part.

Preferably, the invention relates to a monoclonal antibody or one of its fragments according to the invention, characterized in that the epitope of the OZF protein is situated on the N-terminal part comprising the tyrosine residue situated at position 10 of the sequence of the human OZF protein as described by Le Chalony et al., 1994, in figure 1B, page 400; preferably, said epitope is carried by the fragment having the sequence aa7-aa17 of said human OZF sequence.

In an equally preferred manner, the invention relates to a monoclonal antibody or one of its fragments according to the invention, characterized in that it is capable of recognizing, in addition, the human OZF protein whose sequence exhibits a lysine/arginine polymorphism in position 8 of the sequence of the human OZF protein as described by Le Chalony et al., 1994, in figure 1B, page 400.

Among the monoclonal antibodies according to the invention, there is preferred in particular the monoclonal antibody or one of its fragments characterized in that it is produced by a cell as deposited at the Centre National de Culture de Microorganisme (CNCM) (Institut Pasteur, Paris, France) on 6 September 1999 under the number I-2308.

The hybridoma as deposited at the CNCM on 6 September 1999 under the number I-2308 also forms part of the present invention.

The invention comprises, in addition, a monoclonal antibody or one of its fragments according to the invention, characterized in that it is chosen from the humanized, chimeric or anti-idiotype antibodies.

The humanized monoclonal antibodies according to the invention or fragments thereof may be prepared by techniques known to a person skilled in the art (Carter et al., 1992; Singer et al., 1992 and Mountain et al., 1992). Such humanized monoclonal antibodies according to the invention are preferred for their use in diagnostic methods *in vivo* and therapeutic methods.

The chimeric-type monoclonal antibodies or fragments thereof according to the invention may be produced using genetic recombination techniques. For example, the chimeric monoclonal antibody may be produced by cloning a recombinant DNA comprising a promoter and a sequence encoding the variable region of a monoclonal antibody according to the invention and a sequence encoding the constant region of a human antibody. A chimeric antibody of the invention encoded by such a recombinant gene will be for example a mouse-human chemera, the specificity of this antibody being determined by the variable region derived from the murine DNA and its isotype determined by the constant region derived from human DNA (Verhoeyn et al., 1988).

The monoclonal antibodies or fragments thereof according to the present invention also include anti-idiotype antibodies produced by methods known to persons skilled in the art (Cozenza et al., 1976 and Harlow et al., 1988).

Also included in the invention are the monoclonal antibodies and fragments thereof according to the present invention which are obtained by genetic recombination or by chemical synthesis.

The invention also comprises a monoclonal antibody or one of its fragments according to the invention, characterized in that it is labeled.

The monoclonal antibodies according to the invention or fragments thereof may also be, according to the invention, provided in the form of antibodies labeled so as to obtain a detectable and/or quantifiable signal.

The labeled monoclonal antibodies according to the invention or fragments thereof include for example so-called immunoconjugated antibodies which may be conjugated for example with enzymes such as peroxidase, alkaline phosphatase, β-D-galactosidase, glucose oxidase, glucose amylase, carbonic anhydrase, acetylcholinesterase, lysozyme, malate dehydrogenase or glucose-6-phosphate dehydrogenase or through a molecule such as biotin, digoxigenin or 5-bromodeoxyuridine. Fluorescent markers may also be conjugated with the monoclonal antibodies or fragments thereof according to the invention and include in particular fluorescein and its derivatives, fluorochrome, rhodamine and its derivatives, GFP (GFP for "Green Fluorescent Protein"), dansyl, umbelliferone and the like. In such conjugates, the monoclonal antibodies of the invention or fragments thereof may be prepared by methods known to persons skilled in the art. They may be coupled to enzymes or to fluorescent markers directly or via a spacer group or a linking group such as a polyaldehyde, such as glutaraldehyde, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DPTA), or in the presence of coupling agents such as periodate and the like. The conjugates comprising fluorescein-type markers may be prepared by reaction with an isothiocyanate.

Other conjugates may also include chemiluminescent markers such as luminol and dioxetanes or bioluminescent markers such as luciferase and luciferin.

Among the markers which may be attached to the monoclonal antibody or one of its fragments according to the invention, radioactive markers such as ^{14}C, ^{36}Cl,

^{57}Co, ^{58}Co, ^{51}Cr, ^{152}Eu, ^{69}Fe, ^{3}H, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{75}SE and ^{99m}Tc, which may be detected by known means such as a gamma or scintillation counter, or autoradiography, and the like, are also preferred.

The present invention also comprises labeled monoclonal antibodies or fragments thereof according to the invention in which the conjugate may be a detectable marker chosen from the markers which may be used in imaging application *in vivo*. Examples of such markers according to the invention are ^{72}As, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{123}I, ^{125}I, ^{131}I, ^{111}In, ^{97}Ru, ^{99m}Tc, ^{201}TI and ^{89}Zr.

The term "imaging in vivo" should be understood to mean, in the present description, any method allowing the detection of a labeled monoclonal antibody according to the present invention or one of its fragments which specifically bind to the epitope of the OZF protein in the patient's body. The patient will be preferably a man who is likely to have tumor cells abnormally expressing the OZF protein.

The present invention also comprises the labeled monoclonal antibodies or fragments thereof according to the invention in which the conjugate may be a marker chosen from paramagnetic markers which may be used in imaging application *in vivo*. Such markers according to the invention are for example paramagnetic isotopes which are particularly used in magnetic resonance imaging (MRI) and which include in particular ^{62}Cr, ^{162}Dy, ^{56}Fe, ^{157}Gd and ^{55}Mn.

As mentioned above, for the preparation of monoclonal antibody conjugates, the isotopic or paramagnetic marker may be attached to the antibody according to the invention or one of its fragments either directly or indirectly using an intermediate functional group. According to the invention, the monoclonal antibody or one of its fragments may be labeled by any technique known to persons skilled in the art such as for example those described by Wagner et al., 1979 and Saha et al., 1976. The radiolabeled

monoclonal conjugates according to the present invention may be for example iodinated by contact with sodium or potassium iodide and a chemical oxidizing agent such as sodium hypochlorite or an enzymatic oxidizing agent such as lactoperoxidase.

The type of detection of the instrument used will be a major factor in the selection of the marker. For example, the radioactive or paramagnetic isotopes will be chosen, in particular for imaging *in vivo*, according to the type of instrument used which will determine the selection of these markers. Preferably, the radioactive markers chosen should have a period which is detectable by the type of instrument chosen. These monoclonal antibodies may thus be used for example as imaging agent, *in vivo* or *ex vivo*, on biological samples in any conventional method which makes it possible to visualize by an image a diagnosis of a pathological condition linked to the abnormal expression of the OZF protein. These imaging agents or the reagents for diagnosis *in vitro*, characterized in that they comprise a labeled monoclonal antibody according to the invention, as well as their uses in methods of diagnosis *in vitro* or of diagnosis by imaging *in vivo* for the diagnosis of a pathological condition linked to the abnormal expression of the OZF protein indeed form part of the present invention.

The invention also comprises a monoclonal antibody or one of its fragments according to the invention, characterized in that it is coupled to a cytotoxic compound.

The cytotoxic agents which may be conjugated with the monoclonal antibodies according to the invention include in particular, but without being limited thereto, alkylating compounds such as mechlorethamine, triethylene phosphoramide, triaziquone, camustine, semustine, methotrexate, mercaptopurine, cytarabine, fluorouracil, antibiotics such as actinomycin, hormones or antagonist hormones

such as corticosteroids, such as prednisone or progestins. The monoclonal antibody conjugates according to the invention may be prepared by conjugating cytotoxic substances containing either the intact toxin or their derived A chain with the monoclonal antibody or one of its fragments using techniques by persons skilled in the art (Chaudry et al., 1993; Sung et al., 1993 and Selvaggi et al., 1993).

The monoclonal antibody according to the invention may also be a heteroconjugated monoclonal antibody such as a hybrid molecule composed of two or more antibodies. A heteroconjugate includes for example a single-chain monoclonal antibody according to the invention and a single-chain monoclonal antibody which is specific for a cellular receptor (Kerr et al., 1990 and Hsieh-Ma et al., 1992).

According to another aspect, the subject of the invention is a pharmaceutical composition for the treatment, prevention or diagnosis *in vivo* of a pathological condition linked to the abnormal expression of the OZF protein, characterized in that it comprises:

a) a monoclonal antibody or one of its fragments, according to the invention; and

b) a pharmaceutically acceptable excipient.

Preferably, the pharmaceutical composition according to the invention is characterized in that said pathological condition is chosen from cancers, in particular pancreatic, colon or breast cancer.

The subject of the invention is also the use of a monoclonal antibody or one of its fragments according to the invention for the manufacture of a medicament intended for the prevention or treatment of cancer, in particular pancreatic cancer, colon cancer or breast cancer.

In general, the dose of labeled monoclonal antibodies or one of the fragments thereof for the

diagnosis *in vivo* of a pathological condition linked to the abnormal expression of the OZF protein can vary according to parameters such as age, condition, sex, extent of the disease in the patient, contraindications if they exist, concomittant therapies or other variables to which persons skilled in the art will be able to adjust.

The administration of these compounds to the patient may be local or systemic and may be accomplished intravenously, intra-arterially, by means of the spinal fluid, and the like. The administration may also be performed intradermally or by the intracavity route, orally or nasally, depending on the part of the body which has to be examined. After a sufficient time allowing the monoclonal antibody to bind to the OZF protein, for example 30 minutes to 48 hours, the part of the body which has to be examined is examined by conventional imaging techniques such as MRI, or scintillation imaging. The exact protocol will necessarily depend on specific factors linked to the patient, the part of the body to be examined, the method of administration and the type of markers used. The specific procedures may be determined by persons skilled in the art. The distribution of the bound radioactive isotopes and their decrease over time will then be recorded and monitored. By comparing the results obtained with those obtained for clinically normal individuals, the presence and the location of tumor cells can be determined and monitored.

The quantity of monoclonal antibodies contained in the pharmaceutical compositions according to the present invention and necessary for an effective therapy will depend on various factors such as the mode of administration, the part of the body targeted, the physiological state of the patient, the administration of other medicaments, potential side effects and the like. The dosage for such therapeutic preventions or treatments will be determined so as to optimize its

safety and its efficacy. In general, the dosages used *in vitro* can provide a guide for the quantities used for the administration of monoclonal antibodies *in situ* and thus animal models may be used to determine the effective quantities of monoclonal antibodies according to the invention for the treatment of a particular pathological condition. These methods are in particular discussed in manuals such as Gilman et al. (1990) and Remington's Pharmaceutical Sciences (1990) for methods of administration such as the methods by the oral, intravenous, intraperitoneal, intramuscular or transdermal route. The pharmaceutical acceptable excipients include in particular water, saline solutions, buffers or any other compound described for example in the Merck Index.

The invention comprises, in addition, the use of a monoclonal antibody or one of its fragments for the treatment, the prevention or for the diagnosis *in vitro* or *in vivo* of a pathological condition linked to the abnormal expression of the OZF protein, in particular for the treatment, prevention or diagnosis of cancer such as pancreatic, colon or breast cancer, said use being included in the invention.

The invention also relates to a method for the detecting and or assaying the OZF protein in a biological sample, characterized in that it comprises the following steps:

a) bringing the biological sample into contact with a monoclonal antibody according to the invention; and

b) detecting and or assaying the binding of said antibody to the OZF protein contained in the biological sample.

Moreover, the monoclonal antibodies or fragments thereof according to the invention may also be used for the identification, location, in particular tissue or cellular location, and/or assay of the OZF protein, said use being included in the invention.

The monoclonal antibodies or fragments thereof according to the invention also constitute a means of immunocytochemical or immunohistochemical analysis of the expression of the OZF protein on sections of specific tissues, for example by immunofluorescence, by enzymatic, radioactive or gold labeling. They make it possible in particular to demonstrate and quantify the normal or abnormal specific presence of the OZF protein in biological tissues or samples, which makes them useful for the identification and location of the expression of the OZF protein, for the diagnosis of pathological conditions linked to the abnormal presence of the OZF protein but also for monitoring the progress of methods for the prevention or treatment of pathological conditions requiring said detection or said assay.

More generally, the monoclonal antibodies or fragments thereof according to the invention may be advantageously used in any situation where the expression of the OZF protein has to be observed qualitatively and/or quantitatively.

Preferably, the biological sample consists of a biological fluid, such as serum, whole blood, cells, a tissue sample or biopsies of human origin.

Any conventional test or procedure may be used to carry out such a detection and/or assay. Said test may be a competition or sandwich test, or any test known to persons skilled in the art depending on the formation of an antibody-antigen immune complex. Depending on the applications according to the invention, the monoclonal antibody or one of its fragments may be immobilized or labeled. This immobilization may be carried out on numerous supports known to persons skilled in the art. These supports may in particular include glass, polystyrene, polypropylene, polyethylene, dextran, nylon or natural or modified celluloses. These supports may be either soluble or insoluble.

By way of example, a preferred method uses immunoenzymatic processes according to the ELISA, immunofluorescence or radioimmunological (RIA) technique or the like.

The invention also comprises a kit for the determination of the presence of the OZF protein in a biological sample comprising an antibody or one of its fragments according to the invention.

Also entering into the framework of the invention is a kit for the detecting and or assaying the OZF protein according to the invention in a biological sample, characterized in that it comprises the following components:

a) a monoclonal antibody or one of its fragments according to the invention;
b) where appropriate the reagents for constituting the medium appropriate for the immunological reaction;
c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction.

According to a final aspect, the subject of the invention is a method for evaluating the affinity of a compound to be tested for the OZF protein, characterized in that it comprises:

a) bringing a sample containing said OZF protein into contact with
   i) a monoclonal antibody or one of its fragments according to the invention; and
   ii) said compound to be tested; and
b) measuring the quantity of said monoclonal antibody or one of its fragments, said quantity being inversely proportional to the quantity of compounds to be tested which are bound to said OZF protein.

The monoclonal antibodies according to the invention or fragments thereof may also be used in methods *in vitro* for the selection of compounds capable

of binding to the OZF protein with the desired affinity. Thus, the present invention also comprises methods for the selection, by competition, of pharmaceutical compounds in which the monoclonal antibodies of the invention or fragments thereof enter into competition with the compound to be tested which is capable of binding to the OZF protein. In this manner, the monoclonal antibody or its fragments is used to detect the presence of any compound such as a polypeptide or any other organic compound which may have one or more sites for recognizing the epitope of the OZF protein, which is recognized by the antibodies according to the invention. Thus, these compounds, which are identified by the method according to the invention, may be used to occupy these binding sites on the OZF protein and to act as agent which modulates or antagonizes the biological activity of the OZF protein.

Other characteristics and advantages of the invention will emerge in the remainder of the description with the examples and the figures whose legend is represented below.

LEGEND TO THE FIGURES

FIGURES 1A and 1B. Southern blot analysis of pancreatic and primary tumor cell lines.

Figure 1A: Southern blot hybridization of the genomic total DNAs of placenta and of twelve pancreatic adenocarcinoma cell lines, digested with BglII. Three cell lines, AICPC-1, BxPC-3, Su.86.86 exhibit co-amplification of OZF with RAC. PANC-1 exhibits amplification of RAC only.

Figure 1B: Southern blot hybridization of the genomic total DNA of AICPC-1 (figure 1A), of placenta (P) and of twelve pancreatic adenocarcinoma primary tumors, digested with BglII. Comparison of the intensities of the OZF hybridization signals with those obtained after rehybridization with an N-myc probe shows an increase

in the copy number of the gene in the tumor samples T6 and T9.

FIGURE 2. Expression of the OZF mRNA in pancreatic cell lines. The RNA, from the twelve pancreatic adenocarcinoma cell lines analyzed by Southern blotting in figures 1A and 1B, was analyzed by Northern blotting with a cDNA probe containing the OZF open reading frame. AICPC-1, BxPC-3, Su.86.86 having OZF amplified, exhibit a high level of expression. High levels of expression of OZF are observed in the absence of amplification in Capan-1, -2, MIA PaCa-2 and PANC-1. The absence or a very low expression was observed in a sample of normal adult pancreas (Clontech). The corresponding gel, stained before transfer with ethidium bromide, is shown underneath, with the 28S and 18S ribosomal RNA bands.

FIGURES 3A, 3B and 3C. Western blot analysis of pancreatic and primary tumor cell lines. Identical quantities of protein (10 µg), determined by staining of the extracts after SDS gel electrophoresis (not shown) were transferred onto nitrocellulose. The nitrocellulose was then blocked and hybridized with an anti-OZF polyclonal antibody.

Figure 3A: Pancreatic cell lines previously analyzed by Southern and Northern blotting (figure 1A and Figure 2).

Figure 3B: Immunotransfer of OZF of samples of pancreatic adenocarcinoma primary tumors.

Figure 3C: Immunotransfer of OZF of normal human pancreas. The proteins were extracted under the same conditions for the four independent samples of normal pancreas (P1-P4), the line MIA PaCa-1 (MP) and for a pancreatic carcinoma (T6) expressing OZF. The nitrocellulose was hybridized with anti-OZF and anti-pag polyclonal antibodies so as to verify the integrity of the proteins. The anti-pag antibodies are chicken antibodies purified under the same cfonditions as the anti-OZF antibodies.

FIGURES 4A, 4B and 4C. Immunohistochemical detection of OZF in human pancreatic carcinomas. Peroxidase staining was obtained with an affinity-purified anti-OZF antibody. The anti-OZF antibodies show a granular nuclear stain in the AICPC-1 cells transplanted in athymic mice (figure 4A), in an adenocarcinoma expressing OZF (figure 4B), and the absence of staining in healthy pancreas not expressing OZF (figure 4C) (magnification: 1000X).

FIGURE 5. Western blot analysis of crude extracts of human 293 cells not transfected and transfected with an OZF expression vector. The detection of the endogenous OZF protein (on the left) and after transient detection with a vector for expression of the OZF gene (on the right) in triplicate, with an anti-OZF monoclonal antibody and an anti-mouse secondary antibody coupled to the peroxidase, by chemiluminescence.

FIGURE 6. The immunocytochemical labeling of the OZF protein in cells of the AICPC-1 line obtained from a pancreatic adenocarcinoma overexpressing the OZF protein. The cells are cultured on cover glass, fixed with 4% of paraformaldehyde in PBS, and then permeabilized by 0.1% triton X100. The nonspecific sites are saturated with 2% BSA. The cells are incubated for 1 h with the anti-OZF monoclonal antibody (1/200), washed and then brought into contact for 30 min with the second anti-mouse antibody coupled to a fluorochrome, washed and incubated for 4 min with DAPI (0.2 µg/ml) which specifically labels the nuclei (photos on the left). An OZF-specific nuclear labeling is observed (photos on the right). At low magnification at the top and at high magnification at the bottom.

FIGURE 7. Expression of the OZF gene in colon cancers. Western blot analysis.

With the aid of monoclonal antibodies, we examined the expression of the OZF protein in colon cancer. The study was carried out on 16 colic samples. Of the 16 samples, 10 were obtained from a colic tumor (T1 - T3 -

T5 - T6 - T7 - T8 - T9 - T10 - T11 - T12), and 6 from healthy colic mucous membranes (C2 - C4 - C5 - C6 - C8 - C12). Samples T5 and C5 are obtained from the same patient, likewise for T6 - C6, T8 - C8, and T12 - C12. We deposited, in the electrophoreses wells, equivalent quantities of protein for each colic sample, the samples are separated by migration on polyacrylamide gel, and then transferred on to a PVDF membrane. The OZF protein is revealed with the anti-OZF monoclonal antibody and an anti-mouse antibody coupled to peroxidase.

In the four cases in which there were available a healthy colic sample and a cancerous colic sample taken from the same person, we observed a higher accumulation of the OZF protein in the tumor tissue than in the normal tissue.

By comparing the quantity of accumulated OZF protein, we observed that the tumor tissues T1 - T3 - T5 - T7 - T8 - T9 - T10 - T12 have a higher quantity of OZF protein in relation to the healthy tissue.

FIGURE 8. Comparison of the N-terminal amino acid sequences of the OZF proteins according to their origin and their reactivity with the monoclonal antibodies.

Comparison of the N-terminal region of two allels of the human OZF protein (hu-OZF) which differ by a polymorphism at position 8 (K/R) with the OZF protein of murine (mu-OZF) and bovine (bo-OZF) origin. The sequence of the synthetic peptide used in the competitive inhibition experiments is indicated above (PEPTIDE). The tyrosine residue at position 10 is underligned. The sequence of the carrier protein MBP is indicated in small upper case letters for the MBP-OZF protein. The H/C consensus link ("H/C link") precede the first zinc finger corresponds to position 11-17.

* indicates the positions which are perfectly conserved;

° indicates the K/R polymorphism.

The reactivity with the monoclonal antibody 5B8 according to the invention is indicated in the right hand column (+: positive; -: negative).

EXAMPLES

Materials and methods

Tumor samples and cell lines. Eleven pancreatic adenocarcinoma cell lines were obtained from the American Type Culture Collection (Rockville, USA) and cultured as recommended by the supplier. An additional cell line (AICPC-1) was established in the laboratory from the primary tumor from a sixty-year old patient suffering from a pancreatic adenocarcinoma. The cells were cultured in Dubelcco's modified medium supplemented with 20% (vol/vol) fetal calf serum. Tumor tissues were also obtained from patients suffering from a primary carcinoma of the exocrine pancreas (n = 13; 6 men and 7 women).

Southern and Northern blot analysis. The DNA was extracted according to standard methods for cell lines and for frozen tumors (Sambrook et al., 1989). The fragments obtained after digesting the genomic DNA (5 µg) with endonuclease BglI was separated by electrophoresis on 0.7% agarose gel, transferred onto nitrocellulose (BA85, Schleicher and Schuell) and hybridized with probes labeled with P32 (Le Chalony et al., 1996). The tumor cell lines were hybridized with an OZF probe obtained after amplification by PCR with the aid of internal primers so as to generate a fragment of 1 kb containing the entire open reading frame (847-1840). The primary pancreatic carcinomas were hybridized with a cloned XbaI/ScaI fragment (652-1852) (Le Chalony et al., 1994). The RAC probe (2.2 kb) was prepared from a pUC-RAC clone with the aid of universal primers situated on either side of the multiple cloning site (Cheng et al., 1992). The quantitative evaluation of the hybridization signals was carried out with a phosphoimager (BioRad). The

total RNAs were extracted with the aid of a Rneasy kit (Qiagen). For the Northern blot analysis, 10 µg of RNA were deposited on 1% agarose/2.2 M formaldehyde gel, transferred after migration onto nitrocellulose membrane (BA85, Schleicher and Schuell) and hybridized with the XbaI/ScaI probe.

Production of the polyclonal antibodies. The antisera were developed in chickens using the purified OZF protein as immunogen (Ferbus et al., 1996). The IgY immunoglobulins from chicken egg yolk were prepared as described (Akita et al., 1992). The specific antibodies were obtained from anti-OZF IgY's after affinity purification on a column of MBP-OZF coupled to sepharose 4B (Pharmacia) (Ferbus et al., 1996). The specificity of the anti-OZF antibodies was checked by immunoelectrophoresis. A single band of 33 kDa was observed in the nuclear extracts of cells expressing OZF.

Method of preparing the monoclonal antibody. The recombinant fusion protein MBP-OZF produced in E. coli was affinity purified on an amylose cclumn for MBP (Ferbus et al., 1996). Mice were immunized with the purified MBP-OZF protein. The immunization of Balb/c mice was carried out by three subcutaneous injections of 50 µg of purified MBP-OZF protein at two-week intervals. The anti-OZF immune response is monitored by ELISA and immunoblotting using the MBP-OZF protein. Three days before the fusion, 100 µg of MBP-OZF protein are intravenously injected into mice having the highest antiserum titre. After the screening test by ELISA, we selected, for the fusion step, the mice which had the best response. The splenic leukocytes were fused with the cells of the myelomatous line P3X63/8.653 in the presence of 50% polyethylene glycol. After "HAT" selection in crude culture, the hybridomas are individually encapsulated into droplets of biotinylated agarose and labeled with the MBP-OZF protein conjugated with fluorescin isothiocyanate (+) and with the MBP-PAG

protein conjugated with phycoerythrin (-). The suspension of droplets is then analyzed by flow cytometry and only the hybridomas reacting with the MBP-OZF conjugate are isolated and cloned according to the "secretion capture report web" (SCRW) technique (Kenney et al., 1995).

The monoclonal antibodies are purified from the hybridoma culture supernatant by affinity chromatography on protein G Sepharose column (Pharmacia).

The immunoglobulins produced form the majority of the IgG1s.

The monoclonal antibodies specific for the human OZF protein recognizes the latter in the native or denatured state (by ELISA, by Western blotting, by immunohistochemistry or by immunoprecipitation).

ELISA technique for the selection and characterization of the monoclonal antibodies

- Incubation of the 96-well microplates for ELISA in the presence of 50 µl per well of a solution of MBP-OZF or MBP-PAG (control) at 10 µg/ml in 0.05 M carbonate-bicarbonate buffer, pH 9.6, overnight at 4°C.
- Saturation with a 2% bovine serum albumin solution in phosphate-buffered saline pH 7.3 (PBS).
- Addition of 25 µl of crude hybridoma culture supernatant with 25 µl of a solution of 0.1% Tween in PBS buffer and incubation for 2 hours at 37°C.
- After washing, addition of 50 µl of a 1/10 000 solution of anti-mouse goat antibodies conjugated with alkaline phosphatase (Sigma, St Louis, MO) and incubation for 1 hour at 37°C.
- Detection with a p-nitrophenyl phosphate solution at 1 mg/ml in diethanolamine buffer, pH 9.8, 0.5 mM $MgCl_2$.
- Measurement of the absorbance at 405 nm with a microplate reader (Titertek Multiscan from Labsystem, les Ulis, France).

The isotypes of the monoclonal antibodies are determined by ELISA using a kit for murine hybridoma subtyping (Boehringer-Manneheim, Germany).

The recognition epitope is situated at the level of its NH_2-terminal end. It indeed recognizes the truncated recombinant OZF protein possessing only the first two zinc fingers, and does not recognize the homologous murine protein which differs from the human protein at the level of its first fifteen amino acids.

Extraction of the proteins and Western blot analysis with polyclonal antibodies.

The frozen tumor samples and the cell extracts were solubilized in SDS buffer in the presence of β-mercaptoethanol and heated at boiling temperature for 5 minutes. Equal quantities of proteins (10 µg) were loaded onto 12% SDS-polyacrylamide gel and transferred after migration onto PDVF membrane (Amersham). The membranes were hybridized with the anti-OZF antibodies and then with anti-chicken rabbit antibodies conjugated with alkaline phosphatase (Sigma), diluted 200- and 15 000-fold respectively. The chemiluminescence was produced by incubating the membrane with CDP star (Dupont) and the protein was revealed by autoradiography on an X-OMAT AR5 film.

Extraction of the proteins and Western blot analysis with monoclonal antibodies. The cells are cultured to confluence, trypsinized, concentrated by centrifugation and lyzed with buffer (65 mM Tris-HCl, pH 6.8, 0.01% bromophenol blue, 5% β-mercaptoethanol, 10% glycerol, 2% SDS) in the presence of protease inhibitors, heated to boiling temperature and then sonicated. 50 µg of cellular extract or 0.05 µg of recombinant protein are loaded into each well. The proteins are separated by electrophoresis on a 12% polyacrylamide gel in SDS and transferred onto PVDF membrane (Immobilon-P, Millipore). The efficiency of the protein transfer is checked by Ponceau S staining. The Western blot analysis is carried out according to the technique

described in Ferbus et al., 1996. The blots are hybridized for 2 hours with anti-OZF antibodies diluted 1/500 and then with anti-mouse goat IgGs conjugated with alkaline phosphatase diluted 1/10 000. The same conditions are used for the positive control with chicken anti-OZF antibodies diluted 1/200 and rabbit anti-chicken IgY antibodies 1/10 000. The alkaline phosphatase is detected by chemiluminescence with the CDP-star reagent (NEN Live Science Products) and visualized by autoradiography (X-OMAT AR, Kodak, Rochester, NY).

Immunofluorescence. The cells are cultured in Lab-Teck chamber slides (Nunc Inc., Naperville, IL) for 24 hours. The Lab-Teck slides are washed twice in PBS and fixed with a 4% paraformaldehyde solution in PBS (w/v) for 15 minutes at room temperature. The cells are then rinsed in PBS and incubated twice for 30 minutes in a solution containing 0.1 M glycine, 0.2 M Tris-HCl pH 7.5 and then permeabilized in a solution of triton X100 at 0.1% (v/v) in PBS for 5 minutes. The purified anti-OZF antibodies are incubated for 1 hour at 37°C (dilution 1/100) in PBS buffer containing 0.5% bovine serum albumin (w/v). After washing, the cells are incubated for 30 minutes at 37°C with goat anti-mouse IgG antibodies (H+L) conjugated with FITC (fluorescein isothiocyanate) diluted 1/80 (Biosys S.A., France). The slides are counterstained with DAPI (4'6-diamidino-2-phenylindole) for staining the chromatin DNA, fixed with a solution (Vectashield, Vector Laboratories, Inc. Burlingame, CA) and observed under a fluorescence microscope.

Immunohistochemical labelling of OZF. The staining was obtained by a peroxidase-coupled anti-chicken antibody after incubation with a purified anti-chicken OZF antiserum, on frozen tissue sections (5 µm) as described (Terris et al., 1995).

Transfection of NIH3T3 cells. The overexpression of OZF is obtained by transfecting a murine cell line NIH3T3

with a plasmid vector expressing the OZF protein in eukaryotic cells. The culture medium is inoculated with a cell density of 0.8×10^6 cells in Petri dishes 60 mm in diameter and then the cells are transfected 24 h later with 2 µg of plasmid using the calcium phosphate technique. 5 to 6 h after the transfection, the precipitates are removed and the cells are resuspended in culture medium. The next day, the cells are transferred into Lab-Tek chamber slides and fixed before analysis by immunofluorescence.

EXAMPLE 1: Amplification of the OZF gene in the pancreatic adenocarcinoma lines

To evaluate the copy number of the OZF gene in the cells of the tumor lines and to determine if it belongs to the amplicon carrying the RAC gene, the total genomic DNAs, of 12 cell lines and of a placenta, were digested with the restriction enzyme BglII, separated on agarose gel and hybridized by Southern blotting with OZF and RAC DNA probes. As shown in figure 1A, three lines AICPC-1, BxPC-3, SU.86.86 exhibit an amplification signal in a BglII genomic fragment of 5.3 kb in size. The quantification of the amplification levels reveals that the OZF gene is approximately 60 copies, 6 copies and 30 copies in AICPC-1, BxPC-3, SU.86.86 respectively. Co-amplification with RAC is observed in these three lines; however, PANC-1 shows amplification of RAC alone. The number of copies of the RAC and OZF genes is similar in BxPC-3. The relative intensity of the amplification signal is higher for the OZF gene than for RAC in AICPC-1 and lower in SU.86.86. These experiments show that the OZF gene is co-amplified with RAC in three of the four cell lines. Contrary to what had been reported, the amplification of RAC in the AsPC-1 line is not detected (Cheng et al., 1996; Miwa et al., 1996). The karyotype analyses of the cell lines confirmed the ploidy and the specificity of the

chromosome labeling of each line including AsPC-1 (Curtis et al., to be published). The amplification previously described in AsPC-1 may be due to a subclone or may have been lost during propagation in culture.

The state of amplification of OZF was examined in tumor samples. Twelve pancreatic primary adenocarcinomas, taken randomly, were analyzed by Southern blotting with an OZF probe (figure 1B). Two tumors (T6 and T9) exhibit an increase in the hybridization signal (3 to 5 times) compared with the placental DNA, after normalizing with the signal obtained after rehybridization with a single copy of the N-myc gene. This signal is considered to be significant for the amplification given the high proportion of nontumor cells in the tumor tissues.

EXAMPLE 2: Expression of OZF in the pancreatic lines and in the tumor samples

The expression of OZF was examined first in the tumor lines by Northern blotting (figure 2). Various levels of expression were detected in all the lines. The lines AICPC-1 and SU.86.86, which exhibit the highest amplification levels, express the highest levels of OZF mRNA. Moderate expression is observed in BxPC-3, Capan-1, Capan-2, MIAPaCa-2 and PANC-1, and a lower expression is found in the remaining four lines.

The expression of OZF was also evaluated at the protein level by Western blotting using a polyclonal antibody directed against the recombinant OZF protein expressed in E. coli (Ferbus et al., 1996). The OZF protein was detected in all the cell lines (figure 3A). The highest level of the protein is found in the cell lines which exhibit amplification of the OZF gene (AICPC-1, BxPC-3 and SU.86.86). As the pancreatic tissue presents RNAs which are often degraded, the expression of OZF was studied in tumors and the normal pancreas by determining the accumulation of its protein. To compare the level of the OZF protein in the normal adult pancreas with those found in the tumors,

the proteins of four different pancreatic samples were extracted according to the standard protocol and analyzed by Western blotting (figure 3C). The integrity of the extracts was checked using a polyclonal antibody directed against the ubiquitous PAG protein of 22 kDa which is expressed in proliferating cells (Prospéri et al., 1993). Among the eight samples of primary pancreatic adenomas studied, three showed a low level of OZF protein as in the samples of healthy pancreas. Five tumors (T6-T9, T12), exhibit the highest levels of OZF (figures 3B and 3C). Consequently, the OZF gene is expressed at high levels in more than half of the adenocarcinomas analyzed.

EXAMPLE 3: Restriction of the expression of OZF to tumor cells in pancreatic tumors

The possible use of anti-OZF antibodies in immunohistochemical studies was determined. The expression of OZF was detected (figure 4A) in a tumor obtained after transplantation of AICPC-1 cells in athymic mice. The immunohistochemistry was performed on frozen pancreatic carcinoma sections so as to determine if other types of cells, in addition to the tumor cells, contribute to the expression of OZF. One example of pancreatic carcinoma showing an expression of OZF is shown in figure 4B. The anti-OZF antibodies reveal, on staining, a nuclear granulation which is predominant in the tumor cells. The other types of cell such as the fibroblasts and the endothelial cells exhibit a very weak or an absence of staining. Thus, in pancreatic tumors, the expression of OZF is restricted mainly to the tumor cells and the anti-OZF antibodies may be used to detect the tumor cells in heterogeneous pancreatic tumors.

EXAMPLE 4: Expression of the OZF gene in colon cancers

With the aid of monoclonal antibodies, the expression of the OZF protein was examined in colon cancers. The study was carried out on 16 colic samples. Of the 16 samples, 10 were obtained from a colic tumor

(T1 - T3 - T5 - T6 - T7 - T8 - T9 - T10 - T11 - T12), and 6 from healthy colic mucous membranes (C2 - C4 - C5 - C6 - C8 - C12). Samples T5 and C5 are obtained from the same patient, likewise T6 - C6, T8 - C8 and T12 - C12.

Western blot analysis:

Equivalent quantities of protein for each colic sample are deposited in the electrophoresis wells. The samples are separated by migration on a polyacrylamide gel and then transferred onto a PVDF membrane. The OZF protein is revealed with the anti-OZF monoclonal antibody and an anti-mouse antibody coupled to peroxidase. The result is presented in figure 7.

In the four cases where the healthy colic sample and the cancerous colic sample are removed from the same person, a higher accumulation of the OZF protein is observed in the tumor tissue than in the normal tissue.

By comparing the quantity of OZF protein accumulated, it is observed that the tumor tissues T1 - T3 - T5 - T7 - T8 - T9 - T10 - T12 have a higher quantity of OZF protein in relation to the healthy tissues.

From the results, 50% of the cancerous tissues analyzed exhibit a strong overexpression of OZF and 80% of the tumor samples analyzed have a level of expression of the OZF protein which is higher than that observed in the normal tissues. These observations show that the OZF gene is overexpressed in colon cancers.

The results obtained in the preceding examples show that the amplification of the OZF gene was observed in 3 of the 12 pancreatic adenocarcinoma lines (AICPC-1, BxPC-3 and SU.86.86). The increase in the copy number of the gene was also found in 2 of the 12 primary tumor samples (T6 and T9), although levels which are lower than those observed in the lines. Because the pancreatic primary tumors generally contain a large excess of normal cells, hybridization of the

signal by Southern blotting may have been attenuated in tumors T6 and T9. Furthermore, the samples containing few tumor cells, exhibiting a moderate amplification signal, escape detection. Xenotransplants in athymic mice ought to allow better estimation of the amplification of the OZF gene in primary pancreatic tumors. In addition to these results, the amplification of OZF was identified by fluorescence in situ hybridization in the tumor used to generate the AICPC-1 line (Curtis et al., to be published). Thus, the amplification of OZF takes place in the primary pancreatic tumors and in the cell lines.

All the pancreatic adenocarcinoma lines express the OZF protein and mRNA but at different levels. The highest levels of OZF mRNA were found in the lines which amplified the OZF gene (AICPC-1, BxPC-3 and SU.86.86). Although the estimation of the protein may be less accurate, the level of the OZF protein was compared in the two primary tumors exhibiting amplification of the copy number (T6 and T9), with the cell line MIAPaCa-2. The two tumors express high levels of OZF proteins. Thus, the OZF gene which is highly expressed in all pancreatic tumors exhibiting amplification is not inactivated in the amplicon 19q13.1.

Gene amplification is an important mechanism for increasing the expression of genes involved in carcinogenesis even if overexpression is often observed in the absence of amplification. The studies of expression of four independent samples of normal pancreas and of three samples of primary tumors show very low or undetectable levels of OZF proteins. By contrast, all the lines express OZF and four of them show high levels of OZF mRNA in the absence of amplification. The most significant case is that of the PANC-1 line which possesses two copies of the OZF gene and expresses a high level of mRNA 2 to 4 times lower than that of the SU.86.86 line which possesses 30

copies of the OZF gene. High levels of OZF protein are also observed in primary tumors without amplification of the OZF gene, and immunohistochemistry shows that the OZF protein is essentially detected in the tumor cells. Apart from the gene amplification status, OZF is overexpressed in 7 of the 12 tumor lines and in 5 of the 8 primary tumors. The highest levels of expression of OZF in a large sample of tumors compared with the normal pancreas suggest that this gene is involved in the oncogenic process. Furthermore, it could be used as a marker for detecting rare tumor cells in the pancreas.

Outside the OZF gene, the q13.1 region of chromosome 19 comprises several candidate genes. The best characterized is the RAC gene, located in the vicinity of OZF, which encodes a serine-threonine kinase (The Human Gene Map, 1997). The two genes are co-amplified in the cell lines; however, only the RAC gene was found amplified in PANC-1. However, in the PANC-1 line, in which the RAC gene is amplified, the mRNA levels are only slightly lower than those observed in the cell lines which exhibit amplification of the OZF gene. This cell line also contains an abundant OZF protein. Thus, the overexpression of OZF in PANC-1 may be due to a mechanism other than gene amplification as in the case of other known genes. For example, in breast and ovarian cancers, the prevalence of HER2/neu overexpression occurs more frequently than the amplification and was used to predict the rate of survival (Slamon et al., 1989).

Example 5: Selection of the hybridomas and characterization of the monoclonal antibodies

A. Selection of the hybridomas

After immunization and then cell fusion, the antibodies secreted by a single hybridoma are examined using the SCRW technique and selected one by one by flow cytometry. 22 supernatants reacting by FACS

(fluorescence-activated cell sorter) with the recombinant OZF protein and not reacting with the carrier protein MBP are tested for their activity with the recombinant protein MBP-OZF. Half of the cell lines are positive by ELISA and immunoblotting (cf. table 1). No reactivity is observed after ELISA on the MBP-PAG protein, indicating that these supernatants are specific for the OZF polypeptide (Prospéri M-T et al., 1993). Since some monoclonal antibodies can react with epitopes resulting from the fusion with the carrier protein MBP, the reactivity of the supernatants was examined with the OZF protein produced by eukaryotic cells. After analysis by immunoblotting or immunofluorescence in the presence of endogenous OZF proteins derived from human mammary cells HBL 100, six monoclonal antibodies were selected on the basis of their high reactivity with the human OZF protein (cf. table 1 below).

TABLE 1: Screening and selection of the anti-OZF monoclonal antibodies

CELL LINE	ELISA MBP-OZF	ELISA MBP-PAG	IMMUNO-BLOTTING MBP-OZF	IMMUNO-BLOTTING HBL 100 CELLS	IFA HBL 100 CELLS
4A5	+	-	+	-	-
5B1	-	-	-	-	-
5B8	+++	-	+++	+++	++
5B9	+	-	+	-	+
4C3	-	-	-	-	-
4C8	+	-	+	-	-
5C10	+++	-	+++	+++	+++
5C11	+++	-	-	-	+
5D9	+++	-	+++	-	++
5D10	+	-	-	-	-
5D11	+	-	-	-	-

CELL LINE	ELISA MBP-OZF	ELISA MBP-PAG	IMMUNO-BLOTTING MBP-OZF	IMMUNO-BLOTTING HBL 100 CELLS	IFA HBL 100 CELLS
5E9	++	-	++	+	+
5E10	+++	-	-	-	+
5F1	+	-	-	-	-
5F4	+	-	-	-	-
5F8	+	-	-	-	-
5G10	+++	-	+++	+++	+
5H2	-	-	-	-	-
5H4	+++	-	+++	-	-
5H7	+++	-	+++	+++	+++
5H8	+++	-	++	-	+
4H10	+++	-	+++	+++	+++

Legend to Table 1: The crude culture supernatants of 22 hybridoma clones selected by the SCRW technique were tested successively by ELISA against the recombinant proteins MBP-OZF and MBP-PAG, and then by immuno-blotting and immunofluorescence (IFA) against the protein MBP-OZF and the endogenous OZF protein derived from human cells HBL 100. The recombinant protein MBP-PAG was used as negative control.

B. Characterization of the monoclonal antibodies

After purification on protein G Sepharose column, the immunoglobulin subtypes of the six monoclonal antibodies selected were determined. Five antibodies (5B8, 5C10, 5G10, 5H7, 4H10) belong to the IgG1 subtype and one antibody to the IgG3 subtype (5D9). The monoclonal antibodies were also tested by immunoblotting and immunofluorescence with various extracts of mammalian cells. Western blot analysis on the human extracts (cells of the breast, kidney, colon and pancreas) detect a single band of 33 kDa characteristic of the OZF protein. Unexpectedly, the monoclonal antibodies do not recognize the bovine and

murine OZF proteins in spite of their strong identity (95%) with the human OZF protein (Le Chalony, C. et al., 1996; Blottière L. et al., 1999). The absence of reactivity does not result from the translation process since the murine OZF proteins translated in vitro are not detected either. The same results are observed by immunofluorescence (cf. table 2 below), a strong nuclear expression of OZF is observed after transfection of the murine cells NIH3T3 with the expression vector. No fluorescence is visible on the nontransfected murine NIH3T3 cells. Consequently, the six monoclonal antibodies thus produced and characterized according to the invention are highly specific for the human OZF protein.

EXAMPLE 6: Determination of the epitope

Since the monoclonal antibodies react only with the human OZF protein, the human, bovine and murine OZF sequences were compared so as to determine, in a first stage, the location of the epitope.

Most of the variable domains are situated in the first three zinc fingers and, in the sequence of the leader peptide (PL) of 10 amino acids, precede the zinc finger domain (Le Chalony et al., 1996; Blottière et al., 1999).

This first stage suggests that the location of the epitope is plausibly situated in the N-terminal part of the OZF protein. Given the fact that the protein MBP-OZF, for which the first five amino acids of the OZF protein are missing, reacts with the monoclonal antibodies according to the invention, it is highly probable that the epitope against which these monoclonal antibodies are directed is situated at the junction between the leader peptide and the first zinc finger (cf. figure 8).

To confirm this hypothesis, a competitive inhibition ELISA technique is carried out in the presence of a peptide whose sequence corresponds to

fragment aa4-aa17 of the human OZF protein. This peptide proves to be a potent competitive inhibitor toward the monoclonal antibodies according to the invention (cf. table 2 below).

TABLE 2: Characterization of the monoclonal antibodies selected

Method of detection	**Antigen**	**Antibody**						
		Y-OZF	**5B8**	**5C10**	**5D9**	**5G10**	**5H7**	**4H10**
ELISA	Monoclonal antibody	IgY	Ig1	Ig1	Ig3	Ig1	Ig1	Ig1
	MBP-OZF	+	+	+	+	+	+	+
	MBP-OZF + Competition by peptide aa4-aa17	+	-	-	-	-	-	-
Immunoblotting	Human OZF	+	+	+	+	+	+	+
	Bovine OZF	+	-	-	-	-	-	-
	Murine OZF	+	-	-	-	-	-	-
	Murine OZF translated in vitro	+	-	-	nd	nd	-	nd
Immunofluorescence	Human OZF	+	+	+	+	+	+	+
	Murine NIH3T3 cells	+	-	-	-	-	-	-
	Murine NIH3T3 cells transfected with human OZF	+	+	+	+	+	+	+

Legend to Table 2: Six anti-OZF monoclonal antibodies were tested by ELISA, immunoblotting and immunofluorescence. The anti-OZF chicken polyclonal antibody and monoclonal antibodies were diluted 1/500 and 1/100, respectively. The competition is obtained in the presence of 0.05 mg/ml of the synthetic peptide aa4-aa17 (cf. figure 8).

For the immunoblotting, the following extracts were used:

- human origin: breast cells HBL 100, kidney cells 293, pancreatic cells SU86-86, colon cells RC8 and TC7;
- murine origin: fibroblasts NIH3T3;
- bovine origin: FBHE cells of the endothelium and LB9THY lymphocytes.

The immunofluorescence was produced on cell lines HBL100 and NIH3T3.

Anti-OZF chicken polyclonal antibody was used as positive control (Y-OZF).

(+): detection of OZF; (-): no detection of OZF; nd: not done.

These results demonstrate that the six monoclonal antibodies according to the invention tested recognize a common epitope of the human OZF protein situated at the junction between the leader peptide and the first zinc finger. In this region, comparison of the human and bovine sequences of the OZF protein demonstrates two substitutions:

- a first conservative substitution at position 8 resulting in a lysine/arginine polymorphism in humans which does not modify the reactivity of the monoclonal antibodies; and
- the substitution of a tyrosine residue by a leucin residue at position 10 in the bovine sequence.

In the sequence of the murine OZF protein, a cysteine residue is found at position 10 and two substitutions at positions 13 and 14.

Consequently, these results show that the presence of a tyrosine residue at position 10 is critical for the recognition of the monoclonal

antibodies and probably influences the conformation of this epitopic region.

Comparison of the sequence of this epitope with sequences of proteins derived from databanks has not made it possible to identify other proteins carrying this epitope.

Thus, six monoclonal antibodies efficient for the detection of the recombinant and native OZF protein by ELISA, western blotting or immunofluorescence have thus been purified and characterized. Five antibodies are of the IgG1 type and one of the IgG3 type. By contrast to the polyclonal antibodies which detect numerous zinc finger proteins other than the human OZF protein, these monoclonal antibodies according to the invention recognize a single epitope of the human OZF protein. The sequence of this single epitope situated at the junction between the first ten amino acids and the zinc finger domain is not present in the other Kruppel proteins, including the OZF proteins of murine and bovine origin. These monoclonal antibodies according to the invention are highly specific for the human endogenous OZF protein, both in its denatured and native form.

Bibliographical references

Akita, E.M. and Nakai, S. Immunoglobulins from egg yolk: Isolation and purification, J. Food Sci., 57: 629-634, 1992.

Blottière L. et al., Cytogenet. Cell Genet. 85: 297-300, 1999.

Carter, et al., PNAS 89: 4285-4289, 1992.

Chaudry, et al., J. Biol. Chem. 268: 9437-9441, 1993.

Cheng, J.Q., Godwin, A.K., Bellacosa, A., Taguchi, T., Franke, T.F., Hamilton, T.C., Tschlis, P.N. and Testa, J.R. AKT2, a putative oncogene encoding a member of a family of protein-serin/threonine kinases, is amplified in human ovarian carcinomas. Proc. Natl. Acad. Sci. USA, 89: 9267-9271, 1992.

Cheng, J.Q., Ruggeri, B., Klein, W.M., Sonoda, G., Altomare, D.A., Watson, D.K. and Testa, J.R. Amplification of AKT2 in human pancreatic cancer cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA, Proc. Natl. Acad. Sci. USA, 93: 3636-3641, 1996.

Cozenza, et al., Eur. J. Immunol., 6: 114, 1976.

Ferbus, D., Le Chalony, C., Prosperi, M.T., Muleris, M., Vincent-Salomon, A. and Goubin, G. Identification, nuclear localisation, and binding activities of OZF, a human protein solely composed of zinc-finger motifs. Europ. J. Biochem., 236: 991-995, 1996.

Geysen, et al. J. Immunol. Methods, 102: 259-274, 1978.

Gilman, et al., Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed. Pergamon Press, 1990.

Harlow, et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Publications pp. 726, 1988.

Hsieh-Ma, et al., Cancer Res., 52: 6832-6839, 1992.

Kenny, et al., Production of monoclonal antibodies using a secretion capture report web. Bio/technology, 13: 787-790, 1995.

Kerr, et al., J. Immun., 144: 4060-4067, 1990.

King, et al., Biochemical J., 290: 723-729, 1991.
Köhler et Milstein, Nature, 256: 495-497, 1975.
Le Chalony, C., Prosperi, M.T., Haluza, R., Apiou, F., Dutrillaux, B. and Goubin, G. The OZF gene encodes a protein consisting essentially of zinc finger motifs. J. Mol. Biol., 236: 399-404, 1994.
Le Chalony, C., Apiou, F., Pibouin, L., Dutrillaux, B. and Goubin G. Constitutive amplification of a zinc finger protein gene in cattle. DNA and Cell Biol. 15: 83-88, 1996.
Miwa, W., Yasuda, J., Murakami, Y., Yashima, K., Sugano, K., Sekine, T., Kono, A., Egawa, S., Yamaguchi, K., Hayashizaki, U. and Sekiya, T. Isolation of DNA sequences amplified at chromosome 19q13.1 -q13.2 including the AKT2 locus in human pancreatic cancer. Biochem. Biophys. Res. Com., 225: 968-974, 1996.
Mountain, et al., Biotechnol. Genet. Eng. Rev., 10: 1-142, 1992.
Prospéri, M.T., Ferbus, D., Karczinski, I. and Goubin G. A human cDNA corresponding to a gene overexpressed during cell proliferation encodes a product sharing homology with amoebic and bacterial proteins. J. Biol. Chem. 268: 11050-11056, 1993.
Remington's Pharmaceutical Sciences, 17th ed., 1990.
Saha, et al., J. Nucl. Med. 6: 542, 1976.
Sambrook, J., Frischt, E.F. and Maniatis, T. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.
Singer, et al., J. Immun. 150: 2844-2857, 1992.
Selvaggi, et al., J. Immuno-therapy, 13: 201-207, 1993.
Skerra et al., Science, 240: 1038-1041, 1988.
Slamon, D.J., Godolphin, W., Jones, L.A., Holt, J.A., Wong, S.G., Keith, D.E., Levin, W.J., Stuart, S.G., Udove, J., Ulirich, A. and Press MF. Studies of the HER-2/neu proto-oncongene in human breast and ovarian cancer. Science, 244: 707-712, 1989.
Sung, et al., J. Cancer Res. 53: 2092-2099, 1993.

Terris, B., Baldin, V., Dubois, S., Degott, C., Fléjou, J.F., Hénin, D. and Dejean, A. PML nuclear bodies are general targets for inflammation and cell proliferation. Cancer Res. 55: 1590-1597, 1995.

The Human Gene Map (1997) The National Center for Biotechnology Information, The National Institutes of Health, Bethesda, USA, http://www.ncbi.nim.nih.gov/SCIENCE96/.

Verhoeyn, et al., BioEssays, 8: 74, 1988.

Wagner, et al., J. Nucl. Med., 20: 428, 1979.

CLAIMS

1. Monoclonal antibody or one of its fragments which is capable of specifically binding to an epitope of the OZF protein.

2. Monoclonal antibody or one of its fragments according to claim 1, characterized in that the OZF protein is of human origin.

3. Monoclonal antibody or one of its fragments according to claim 1 or 2, characterized in that the epitope of the OZF protein is situated on the N-terminal part.

4. Monoclonal antibody or one of its fragments according to claim 3, characterized in that the epitope of the OZF protein is situated on the N-terminal part comprising the tyrosine residue situated at position 10 of the sequence of the human OZF protein.

5. Monoclonal antibody or one of its fragments according to one of claims 1 to 4, characterized in that it is produced by a cell as deposited at the CNCM on 6 September 1999 under the number I-2308.

6. Monoclonal antibody or one of its fragments according to one of claims 1 to 4, characterized in that it is chosen from humanized, chimeric or anti-idiotype antibodies.

7. Monoclonal antibody or one of its fragments according to one of claims 1 to 6, characterized in that it is labeled.

8. Monoclonal antibody or one of its fragments according to one of claims 1 to 6, characterized in that it is coupled to a cytotoxic compound.

9. Cell capable of producing a monoclonal antibody according to one of claims 1 to 5 as deposited at the CNCM on 6 September 1999 under the number I-2308.

10. Pharmaceutical composition for the treatment or prevention of a pathological condition linked to the abnormal expression of the OZF protein, characterized in that it comprises:

a) a monoclonal antibody or one of its fragments, according to one of claims 1 to 6 and 8; and

b) a pharmaceutically acceptable excipient.

11. Pharmaceutical composition for the diagnosis *in vivo* of a pathological condition linked to the abnormal expression of the OZF protein, characterized in that it comprises:

a) a monoclonal antibody or one of its fragments, according to one of claims 1 to 7; and

b) a pharmaceutically acceptable excipient.

12. Pharmaceutical composition according to claim 10 or 11, characterized in that said pathological condition is chosen from cancers, in particular pancreatic, colon or breast cancer.

13. Use of a monoclonal antibody or one of its fragments according to claims 1 to 6 and 8, for the manufacture of a medicament intended for the treatment or prevention of cancer, in particular pancreatic cancer, colon cancer or breast cancer.

14. Use of a monoclonal antibody or one of its fragments according to one of claims 1 to 7, for the diagnosis *in vitro* of a pathological condition linked to the abnormal expression of the OZF protein.

15. Method for the detecting and or assaying the OZF protein in a biological sample, characterized in that it comprises the following steps:

a) bringing the biological sample into contact with an antibody according to one of claims 1 to 7; and

b) detecting and or assaying the binding of said antibody to the OZF protein contained in the biological sample.

16. Kit for the determination of the presence of OZF protein in a biological sample comprising an antibody or one of its fragments according to one of claims 1 to 7.

17. Method for evaluating the affinity of a compound for the OZF protein, characterized in that it comprises:

a) bringing a sample containing said OZF protein into contact with

i) a monoclonal antibody or one of its fragments according to one of claims 1 to 7; and

ii) said compound to be tested; and

b) measuring the quantity of said monoclonal antibody or one of its fragments, said quantity being inversely proportional to the quantity of compounds to be tested which are bound to said OZF protein.

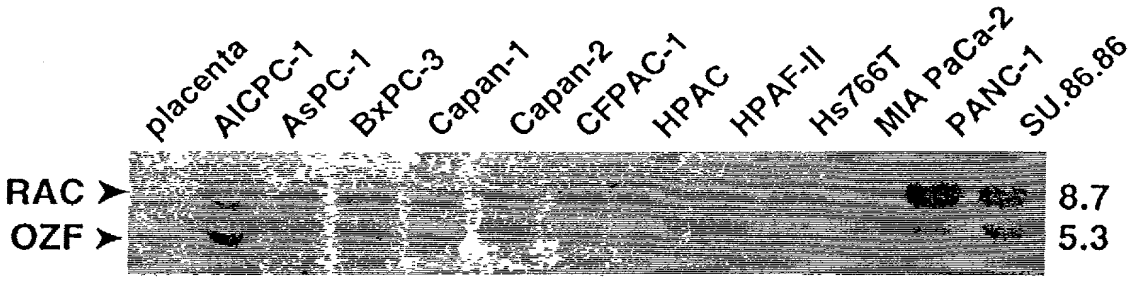


FIGURE 1A

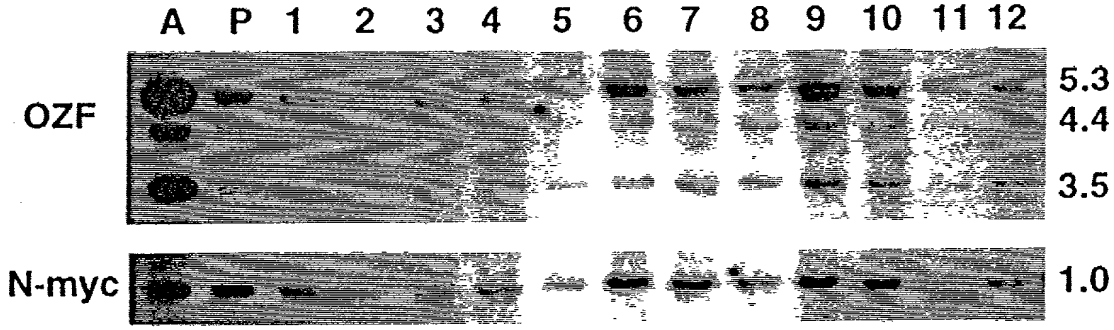
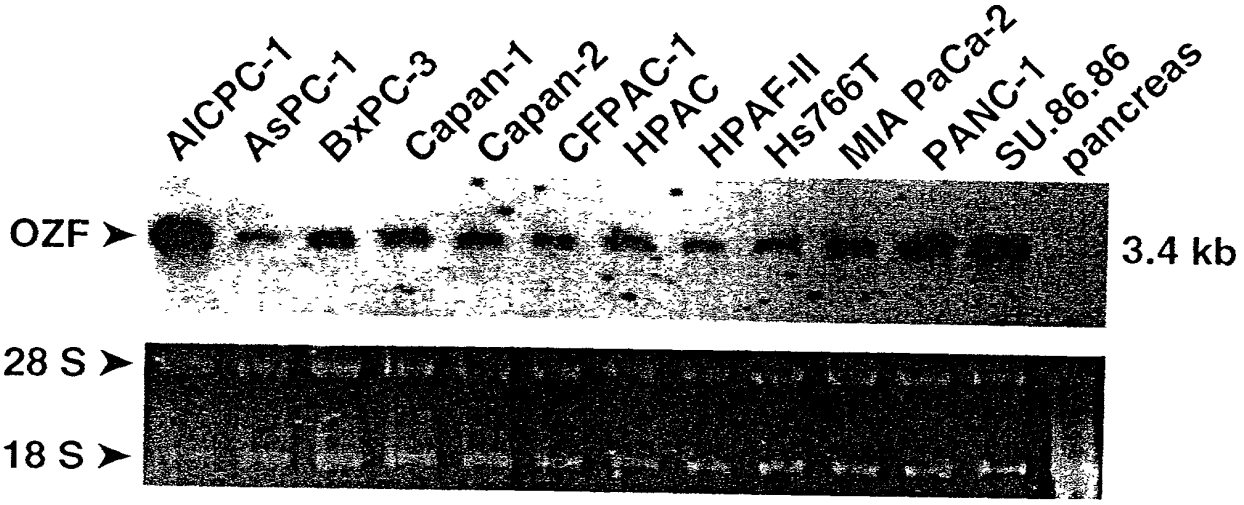


FIGURE 1B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AsPC-1
BxPC-3
Capan-1
Capan-2
CFPAC-1
HPAC
HPAF-II
Hs766T
MIA PaCa-2
PANC-1
SU.86.86
pancreas
OZF
3.4 kb
28 S
18 S

FIGURE 2

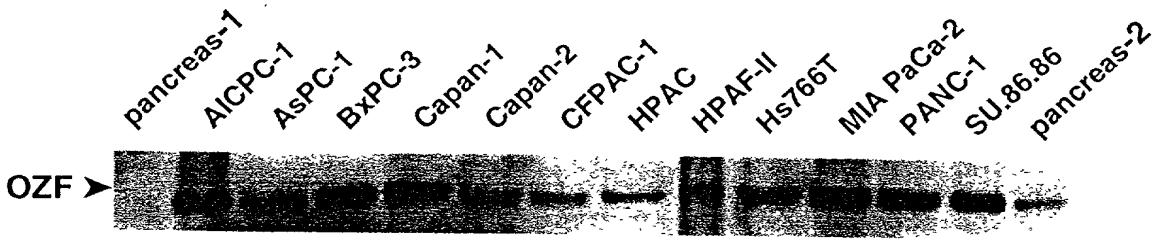


FIGURE 3A

FIGURE 3B

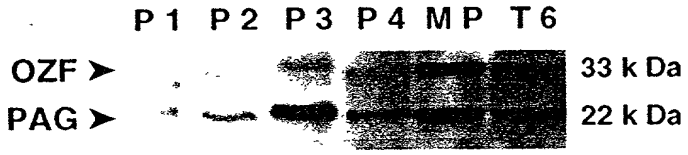


FIGURE 3C

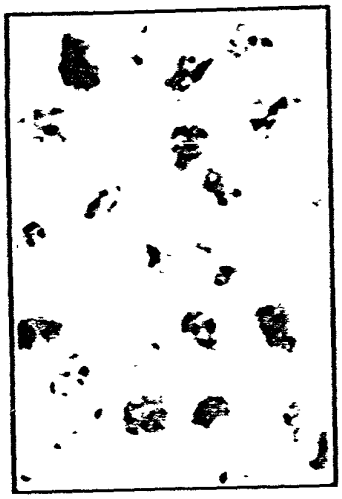


FIGURE 4A

FIGURE 4B

FIGURE 4C

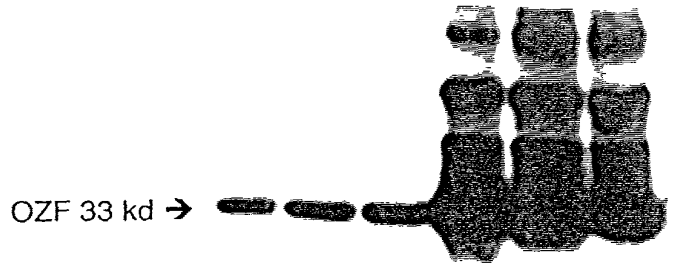
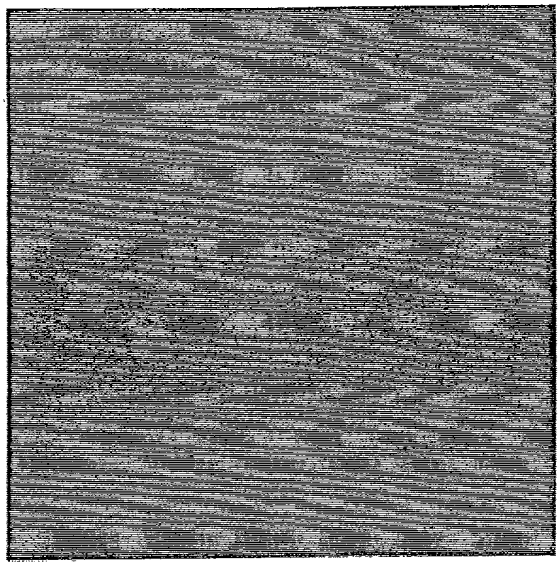
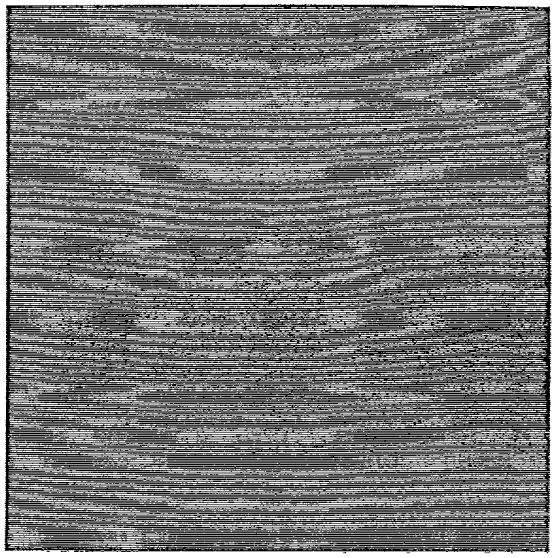


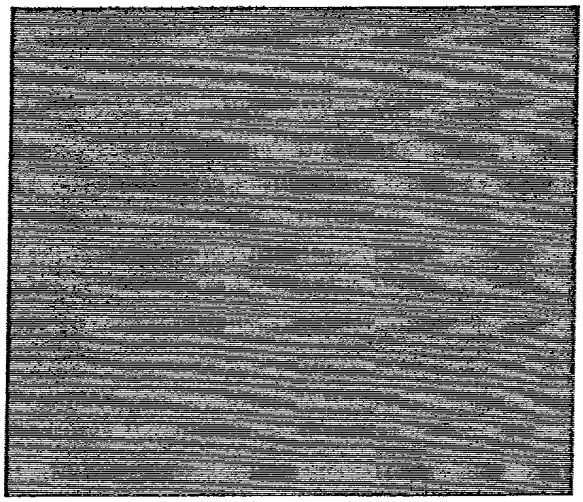
FIGURE 5



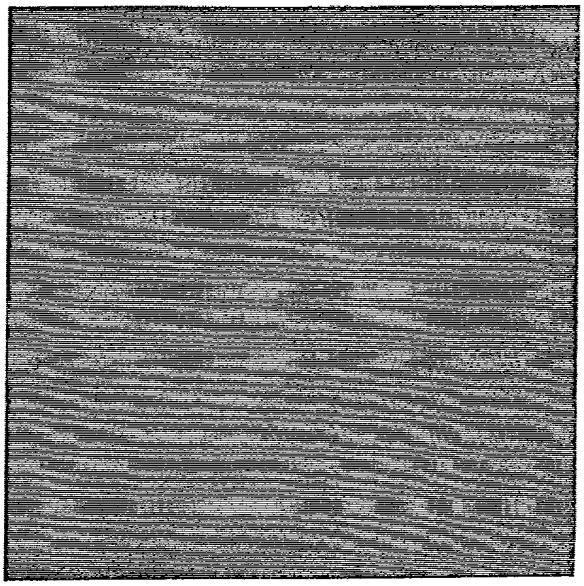
Nuclei labeled with DAPI



Nuclei labeled with anti-OZF



Nuclei labeled with DAPI



Nuclei labeled with anti-OZF

FIGURE 6

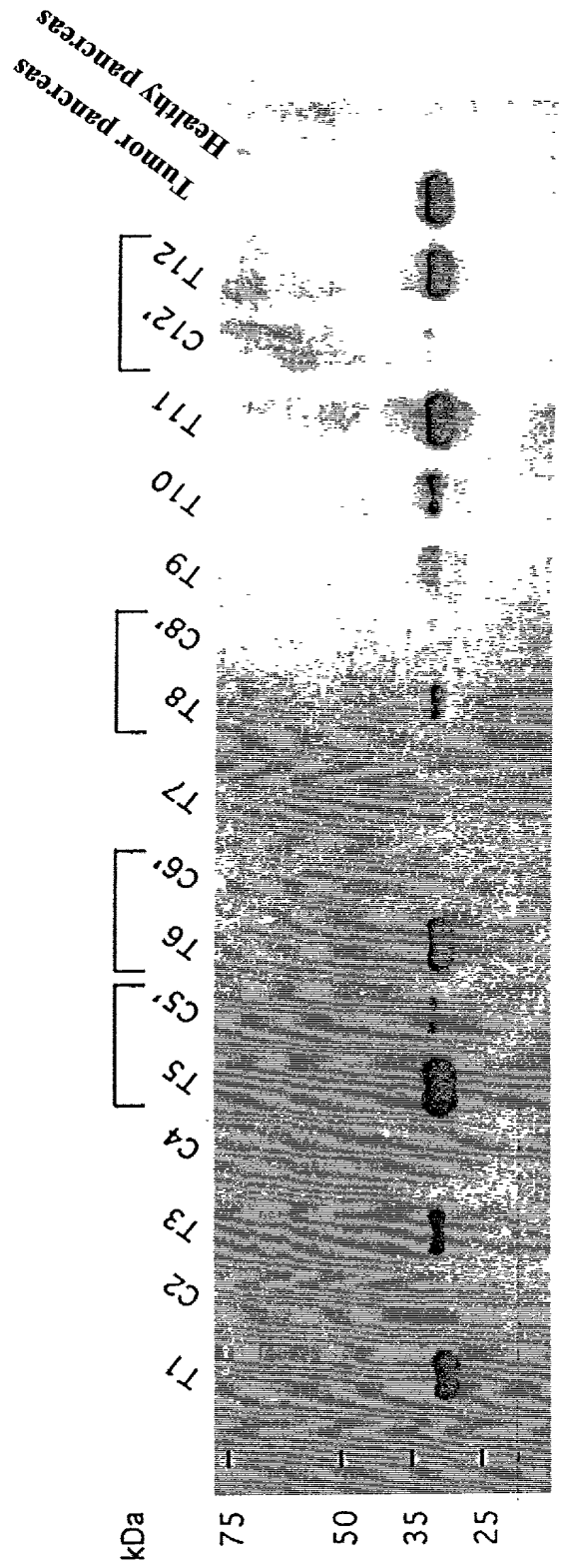

Tumor pancreas
Healthy pancreas
T12
C12'
T11
T10
T9
C8'
T8
T7
C6'
T6
C5'
T5
C4
T3
C2
T1
kDa
75
50
35
25

FIGURE 7

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
PEPTIDE					L	S	Q	Q	K	I	Y	S	G	E	N	P	F A+
MBP-OZF	S	S		R	V	D	L	Q	K	I	Y	S	G	E	N	P	F A+
hu-OZF	M	S		H	L	S	Q	Q	K	I	Y	S	G	E	N	P	F A+
hu-OZF	M	S		H	L	S	Q	Q	R	I	Y	S	G	E	N	P	F A+
bo-OZF	M	S		H	L	S	Q	Q	R	I	L	S	G	E	N	P	F A-
mu-OZF	M	S		H	L	S	Q	Q	R	I	C	S	G	G	S	P	F A-
						*	°	*	*	*		*	*	*			

FIGURE 8

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Gérard Goubin et al.

Entitled: ANTI-OZF PROTEIN MONOCLONAL ANTIBODIES AND THEIR APPLICATIONS IN THE DIAGNOSTIC AND THERAPEUTIC FIELD

Serial No. To be assigned

Filing Date March 8, 2001

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned attorney of record hereby appoints Stephen B. Maebius, Registration No. 35,264 as an associate attorney with full power of association, substitution and revocation, to prosecute the above-identified application and transact all business in the Patent and Trademark Office connected therewith.

Respectfully submitted,

Date March 8, 2001 By _____

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Harold C. Wegner
Attorney for Applicant
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Docket No. _____

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ANTI-OZF PROTEIN MONOCLONAL ANTIBODIES AND THEIR APPLICATIONS IN THE DIAGNOSTIC AND THERAPEUTIC FIELD

the specification of which is attached hereto unless the following box is checked:

☒ was filed on September 8, 1999 as ~~United States Application Number~~ or PCT International Application Number PCT/FR99/02133 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
98/11204	FRANCE	08.09.1998	X

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO.	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No.25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Residence Address		Country of Citizenship
Post Office Address		